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## An acetylated form of histone H2A.Z regulates chromosome architecture in *Schizosaccharomyces pombe*

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### Summary

Histone variant H2A.Z has a conserved role in genome stability, although it remains unclear how this is mediated. Here we demonstrate in fission yeast that the Swr1 ATPase inserts H2A.Z (Pht1) into chromatin and Kat5 acetyltransferase (Mst1) acetylates it. Deletion or unacetyltable mutation of Pht1 leads to genome instability, primarily caused by chromosome entanglement/breakage at anaphase. This leads to the loss of telomere-proximal markers, though telomere protection and repeat length are unaffected by the absence of Pht1. Strikingly the chromosome entanglement in *pht1Δ* anaphase cells can be rescued by forcing chromosome condensation prior to anaphase onset. We show that the condensin complex, required for the maintenance of anaphase chromosome condensation, prematurely dissociates from chromatin in the absence of Pht1. This and other findings suggest an important role for H2A.Z in the architecture of anaphase chromosomes.

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#Equal contribution

### Author Contributions

H-S.K. and V.V. contributed equally to this work. H-S.K. was responsible for the data in Fig. 1 (with A.T. contributing Fig. 1c), Fig. 2 (with Mass Spectrometry help from J.F., T.K., A.E. and J.F.G.), Fig. 5c–d and Supplementary Figs 1 and 3. V.V. (with help from K.G.H.) was responsible for the data in Fig. 4, Fig. 5a–c and Supplementary Figs 2, 3a and 4. S.W. and J.B. performed and analyzed the microarrays in Fig. 3a–b; A.R. and N.J.K. performed and analyzed the genetic screens in Fig. 3c–f. L.R.C. and C.S.B. created the anti-Pht1 antibodies used in Fig. 1. H-S.K., V.V. and M-C.K. planned experiments, analyzed the data and wrote the manuscript.

## Keywords

Chromosome architecture; condensin; H2A.Z; KAT5; RCA; *S. pombe*

## Introduction

The basic repeating unit of chromatin is the nucleosome core particle: 146 bp of DNA wrapped around a core histone octamer composed of a (H3-H4)<sub>2</sub> tetramer and two H2A-H2B dimers. One means of nucleosome-specialization is the replacement of a major histone with a specific variant. These single-copy, non-allelic isoforms are generally expressed throughout the cell-cycle, and many enter nucleosomes through the action of specific deposition machineries 1. Both major and variant histones can be further distinguished by the addition of small chemical moieties, including phosphorylation, acetylation, methylation and ubiquitylation 2,3.

H2A.Z is one of the most studied histone variants. In metazoans it is essential 4, regulating chromosome stability 5, gene activation 6,7 and spermatogenesis 8. The histone is subject to multiple N-terminal acetylations in *Saccharomyces cerevisiae* (*Sc*) 9–11, *Tetrahymena thermophila* 12, and metazoans 13,14. Though the role(s) of these modifications remains uncertain, defects in heterochromatin restriction 11 and chromosome stability 9 are observed when unacetyltable H2A.Z alleles are expressed as the sole source of the histone in budding yeast.

A phenotype common to all H2A.Z-deficient species is genomic instability. This manifests in mammalian cells as chromatin bridges in anaphase 5. Deletion of *Sc* H2A.Z (*htz1Δ*) results in an increased rate of chromosome loss, sensitivity to microtubule destabilizing agents, and synthetic genetic interactions with components of the kinetochore and spindle checkpoint machineries 15. Many of these phenotypes are shared by an unacetyltable *htz1* mutant, suggesting a role for acetylation in genomic stability 9. However we still have little mechanistic understanding of what leads to the chromosome segregation defect in these cells, though centromere organization, kinetochore attachment, cohesin recruitment and arm sister chromatid cohesion appear normal 9.

To explain the role of H2A.Z in chromosome stability we turned to the fission yeast *Schizosaccharomyces pombe* (*Sp*) as its three large chromosomes (*Sc*, 16 × 230kb – 1.3 Mb; *Sp*, 3 × 3.5 – 5.7 Mb) can be more easily monitored during mitosis. We show that *Sp* H2A.Z (*Pht1*; Pseudo-histone 1) co-purifies with a complex almost identical in composition to the *Sc* SWR-C, with this complex required for the insertion of Pht1 into chromatin. Chromatin-associated Pht1 is acetylated on its N-terminus by the KAT5 acetyltransferase (*Sp* Mst1). This modification is essential for Pht1 function, with unacetyltable mutants phenocopying complete deletion of the histone variant in all analyses, including genome-scale genetic-interaction and gene-expression studies. In addition we show that chromosome loss in *pht1* mutants is primarily caused by broad architectural defects and can be suppressed by improving chromosome condensation. Consistent with this we show by Chromatin Immunoprecipitation that the condensin complex is prematurely released from chromatin at anaphase in *pht1Δ* cells.

## Results

### Pht1 is acetylated as a component of chromatin

*Sp* Pht1 has four potential acetyltable lysines (K) on its N-terminus: K5, 7, 12 and 16 (Fig. 1a). We synthesized a peptide containing all four acetyl-lysines and immunized rabbits to

create polyclonal anti-Pht1<sup>Ac</sup>. On immunoblots of *Sp* whole cell extracts (WCEs) this recognized a protein of the appropriate size for Pht1. This signal disappears in WCEs from *pht1Δ*, *-NΔ* (deletion of the N-terminus), or mutants where all four lysines were mutated to unacetyltable arginine (R) or glutamine (Q) (Fig. 1b). Thus the Pht1 N-terminus is acetylated and we have a specific reagent for the modification.

The primary acetyltransferase for *Sc* Htz1 is Esa1, the catalytic subunit of the NuA4 complex 9–11. Esa1 is a member of the KAT5 family, with the most likely *Sp* homolog the essential protein Mst1 16. We created a temperature sensitive (*ts*) allele of Mst1 (*mst1-L271P*), that was slow at the permissive temperature (25°C) and lethal >34°C (Fig. 1c). WCEs from *mst1-L271P* cells showed no appreciable change in total Pht1 levels but a profound reduction in Pht1<sup>Ac</sup> (Fig. 1d), indicating that Pht1 acetylation is Mst1-dependent. Cell fractionation showed that Pht1<sup>Ac</sup> is chromatin-associated, though acetylation is not required for entry to this cellular compartment (Figs. 1e–f).

*Sc* Htz1 associates with the SWR-Complex, containing the Swr1-ATPase, and incorporation of the histone into chromatin is markedly reduced in *swr1Δ* cells 17–19. We subjected WCEs from Pht1.TAP to sequential affinity purification and identified the associated proteins by Mass Spectrometry (Supplementary Table 1). Reciprocal tagging and purification of these factors delineates the *Sp* SWR-C. This is almost identical in composition to the *Sc* SWR-C, even to the level where subunits shared between the *Sp* SWR and Mst1-acetyltransferase complexes are those shared by their *Sc* counterparts, the SWR-C and NuA4 (Fig. 2a). Western analysis with anti-Pht1<sup>Ac</sup> distinguished those subunits of the *Sp* SWR-C required for the efficient acetylation of the histone (Fig. 2b), most likely because of inefficient assembly of the variant into chromatin in each background (Fig. 2c). Thus a pathway first identified in *Sc* also operates in *Sp*: SWR-C inserts Pht1 into chromatin, where it is acetylated by Mst1.

### Acetylation is integral to Pht1 function

To determine the relevance of N-terminal acetylation to Pht1 function, we compared the gene expression and genetic interaction profiles of various unacetyltable mutant alleles (*pht1-NΔ*, *-4KR*, and *-4KQ*) to *pht1Δ*.

The transcription profiles of the unacetyltable mutants were almost identical to *pht1Δ* (e.g. relative P-value:  $\Delta$  to *-4KR* =  $2e^{-195}$ ; Fig. 3a). A striking observation was that almost all genes were modulated to some degree in *pht1* mutants (Fig. 3b), suggesting that the histone acts as a general transcriptional regulator. By comparison *swr1Δ* showed weaker changes in gene expression (Fig. 3b). Since loss of the ATPase strongly reduces, but does not completely ablate chromatin-associated Pht1 (Fig. 2c), it is likely that the remainder is sufficient to mask most H2A.Z-dependent phenotypes.

We then used the Pombe Epistatic Mapper 2 (PEM-2) system to quantitatively analyze genetic interaction patterns 20–21. To this end we individually crossed *pht1Δ*, *-NΔ*, *-4KR*, *-4KQ* and *swr1Δ* strains to a library of 2161 non-essential *Sp* deletions (the Bioneer collection), and derived scores covering each negative (e.g. synthetic sick/lethal) and positive (e.g. suppression) genetic interaction using colony size as a quantitative read-out 20–22–24. Positive interactions enrich for factors that are co-complexed or function in the same pathway 20–22–23. Consistent with this, *pht1Δ*, unacetyltable *pht1*, or *swr1Δ* each gave rise to positive genetic interactions in combination with deletions of all non-essential subunits of the SWR-C (20 and Fig. 3c). This implies that preventing Pht1 acetylation disables the primary function of the SWR-C/Pht1 pathway in fission yeast.

The genetic interaction profile of a particular mutant can be used as a high-resolution phenotype. Comparison of this profile to those generated from other mutants can identify functionally related factors 20·22·23. When compared to data from >100 genetic screens (not shown), *pht1Δ* and *swr1Δ* were highly correlated (Fig. 3d), confirming that they function in the same pathway. Indeed, of all mutants analyzed, the most highly correlated to *pht1Δ* were *-NΔ*, *-4KR* and *-4KQ*, suggesting that N-terminal acetylation is critical for Pht1 function (Fig. 3e). Inspection of individual interactions showed that all unacetylatable *pht1* alleles were synthetic with deletions of factors involved in chromatin modification/remodeling (e.g. COMPASS, RSC, SET3-C), transcription (e.g. Mediator) and chromosome segregation/cytokinesis (e.g. *cut8*, DASH complex) (e.g. Fig. 3f). These are reminiscent of the synthetic interactions displayed by *Sc htz1Δ* 15·17, further suggesting strong conservation of H2A.Z function in each organism.

### Chromosome loss in *pht1Δ* is caused by entanglement at anaphase

Knockout or depletion of H2A.Z in *Sc* 15 or mammalian cells 5 leads to increased rates of chromosome loss. This phenotype was also observed if any component of the *Sp* Pht1<sup>Ac</sup> pathway is disrupted, including mutants in *swr1* (and *msc1*), *pht1* (*pht1Δ*, *-4KR* or *-4KQ*), or *mst1* (Supplementary Table 2 and 16·25·26). One possible explanation for chromosome instability is disruption of the centromere 27. However as in *htz1Δ* cells 9, centromere structure and function appeared normal in *pht1* cells (Supplementary Fig. 1).

On cytological analysis of individual *Sp* cells we observed a >8-fold increase (relative to WT) in the number of *pht1Δ* cells with anaphase chromosome segregation defects (Fig. 4a). Three specific categories of anaphase defects were distinguished using the kinetochore marker Nuf2-GFP: (i) lagging chromosomes, where the mis-segregating chromatin contains at least one kinetochore, (ii) chromosome entanglement, where the mis-segregating chromatin is stretched between the spindle poles but contains no kinetochore, and (iii) entanglement leading to breakage, where broken pieces of chromatin with no kinetochore lag on the spindle (Fig. 4b). The primary segregation defect in *pht1Δ* cells was entanglement: >80% of defective anaphases. This was clearly distinct from the primary anaphase segregation defect in heterochromatin-deficient *clr4Δ* cells: >90% lagging chromosomes (Fig. 4c). Lagging chromosomes result from merotely, a defect where a single kinetochore attaches to microtubules emanating from opposite spindle poles 28. Thus, to a large extent, kinetochore - microtubule attachments appear normal in *pht1Δ* cells, supporting our ChIP observation of a WT-like centromere in *pht1* mutants (Supplementary Fig. 1c). Consistent with this result, *pht1Δ* cells did not activate the spindle checkpoint, nor did they rely on spindle checkpoint genes for survival (not shown).

Chromosome entanglement in *pht1Δ* cells was particularly obvious when we followed telomere segregation at anaphase. To do so, we used the telomere-binding protein Taz1-GFP as a marker. In a normal mitosis, six Taz1-GFP spots (identifying the left and right telomeres on the three *Sp* chromosomes) migrate to opposite poles with the bulk of DNA. However in *pht1Δ* cells we often observed two or more Taz1-GFP foci entangled in the centre, leading to stretched chromosome arms (Fig. 4d). One possible explanation for this entanglement is a defect in telomere function comparable to that observed in cells lacking the protection factor Taz1 29. However many of the phenotypes of *taz1Δ* were not shared by *pht1Δ*: e.g. cold-sensitivity, size of the chromatin bridge, presence of Rad22 foci, *rqh1-SM* suppression (Supplementary Fig. 2). In addition, and most striking, while telomere-repeat length was dramatically increased in *taz1Δ*, it was comparable to WT in *pht1Δ* (Fig. 4e).

To quantify how frequently the entanglement in *pht1Δ* cells leads to chromosome loss, we employed a series of strains containing: (i) lacOperator (*lacO*) repeats integrated at different chromosomal locations, and (ii) LacI-GFP, the fluorophore marked *lacO* binding protein 30.

Surprisingly, marker loss in mitotic *pht1Δ* cells increased with distance from the centromere, suggesting that they generally lost broken pieces of chromosomes rather than whole chromatids (Fig. 4g–h). This is in sharp contrast to *sgo2Δ* cells, which are unable to correct chromosome bi-orientation defects 31–32. In *sgo2Δ* cells marker loss was similar irrespective of chromosome position, suggesting the loss of whole chromatids (Fig. 4h). Together these observations suggest that the primary role of Pht1 in chromosome transmission is to maintain overall chromosome architecture rather than to regulate the function of a specific region (such as the centromere or telomere).

### Pht1 plays a role in chromosome architecture/compaction

Chromosomes condense to a folded rod-shaped structure upon mitotic entry. As the chromatids are pulled to the spindle poles in early anaphase, the chromosome arms trail behind the centromeres, forming a distinctive “arrowhead” structure. This was lost in >50% of cells lacking *pht1* (Fig. 5a). Such a generalized defect further supports the idea that Pht1 regulates overall chromosome architecture rather than that of specific loci.

The primary chromosome segregation defect in *pht1Δ* cells (i.e. chromosome entanglement despite accurate centromere segregation; Fig. 4) is reminiscent of that observed in mutants of condensin, a five-subunit complex instrumental to the architecture and segregation of chromosomes in mitosis 33–36 (Supplementary Fig. 3a). We thus speculated that *pht1* mutants may mis-regulate chromosome condensation during anaphase. If so, it may be possible to suppress their defect by forcing chromosome hyper-condensation. To this end we used the cold-sensitive tubulin mutant, *nda3-KM311*, which fails to assemble microtubules at the restrictive temperature (20°C), leading to a spindle-dependent checkpoint arrest 37. This prolonged early mitotic arrest induces chromosome hyper-condensation 37, and when shifted to the permissive temperature (32°C) these cells enter a synchronous anaphase 31–37. Strikingly, this prolonged mitotic arrest specifically rescued the chromosome segregation defect of *pht1Δ* but not *taz1* cells (Fig. 5b). Arresting *pht1Δ* cells in S-phase with hydroxyurea (HU, depletes nucleotide pools) had no effect on the chromosome segregation defect, showing that merely prolonging the cell-cycle was not sufficient to rescue *pht1Δ* defects (Fig. 5b).

A functional relationship would be indicated by genetic interaction, so we tested that between *pht1* and *condensin*. Unacetylatable *pht1* or *pht1Δ* each raised the restrictive temperature of *ts* alleles of three complex subunits: *cut3-477* (*smc4-S1147P*), *cut14-208* (*smc2-S861P*) and *cnd2-1* (*cnd2-A114T*) (Fig. 5c and Supplementary Fig. 3b). This partial rescue was specific, as *pht1Δ* was synthetic with *rad21-K1*, a mutant in the condensin-related complex cohesin, which holds sister-chromatids together prior to anaphase onset. This suppression is unlikely to be mediated through indirect transcriptional effects (e.g. increased expression of condensin), since the mRNA levels (by gene expression microarray) of all tested complex subunits and known regulators (e.g. Cut17, Ark1, Top2, Fin1, Pim1, Pic1, Acr1, Nuc1) were comparable to WT in *pht1Δ* and unacetylatable *pht1* cells (Supplementary Fig. 3c and not shown). In addition the partial rescue was not observed on deletion of two other transcriptional regulators: the Set1 methyltransferase or Gcn5 acetyltransferase (Fig. 5c).

Our results are consistent with a model where an acetylated form of Pht1 regulates condensin loading and/or localization in mitosis. To test this, we arrested WT or *pht1Δ* cells expressing C-terminally tagged Cut3 (Cut3.HA3) at the G2/M boundary, and then monitored condensin occupancy kinetics through a synchronous mitosis (Fig. 5d). To first ensure that mitotic progression in WT and *pht1Δ* cells followed similar kinetics, we examined the peak septation index and H3-K9<sup>Me2</sup> in each population. A newly formed septum indicates the completion of mitosis and can be visualized by specific staining with Calcofluor



(Supplementary Methods). H3-K9<sup>Me2</sup> is markedly reduced at centromeric repeats (*otr*) during mitosis, increasing as cells enter G<sub>1</sub>/S 38. Each pattern was indistinguishable between WT and *pht1Δ* cells (Fig. 5d). When we examined condensin recruitment, Cut3.HA<sub>3</sub> initially (75 mins) followed similar kinetics at the centromere (*cnt*, *inr* and *otr*) and rDNA of WT and *pht1Δ* cells (Fig. 5d and Supplementary Fig. 3d). However significant differences were then observed: Cut3.HA<sub>3</sub> levels continued to increase in WT cells, peaking at 100 mins before dropping at 125 mins (the latter corresponding to the peak septation index time point; Fig. 5d). However in *pht1Δ* condensin delocalization began by 100 mins, indicating the premature dissociation of the complex from chromatin. This likely explains many of our observations in these cells: loss of the anaphase arrowhead structure leading to chromosome entanglement and the loss of telomere-proximal regions (Fig 5).

## Discussion

In this work we identify and characterize a previously unknown role for the histone variant H2A.Z (*Sp* Pht1) in chromosome architecture at anaphase. In the absence of Pht1, chromosomes frequently entangle in anaphase, which can lead to breakage and loss, particularly of telomere-proximal regions. We provide evidence that chromosome entanglement in *pht1Δ* is most likely due to premature dissociation of the condensin complex in anaphase. We also demonstrate that the factors involved in the chromatin-loading and acetylation of H2A.Z are highly conserved in *Sc* and *Sp*. The last common predecessor of these yeasts was ~ 380 million years ago (mya): by comparison that of the entire mammalian class existed ~ 165 mya 39, while the primate line split to humans and gorillas ~ 8 mya 40. We propose that since the pathways regulating H2A.z localization, modification, and function are so well conserved, the role of the histone variant in chromosome architecture will be equally penetrant.

### Extensive conservation of the Swr1-C

Comprehensive proteomic analyses identify two subunits in the *Sp* SWR-C not seen in its *Sc* counterpart: Msc1 and SPAC4H3.02c (Fig. 2a, Supplementary Table 1, and 41). Both deletions are epistatic with *pht1* (Fig. 3c and 20), further suggesting a functional relationship. Msc1 was originally identified as a multi-copy suppressor of cells defective for checkpoint kinase Chk1 (Rad27) function 42. The Walworth lab have demonstrated an epistatic chromosome loss phenotype in *pht1Δ* and *msc1Δ*, and suggested that Msc1 was upstream of Pht1 in this pathway 26, a relationship our data supports (e.g. Fig. 2c). Msc1 was recently shown to interact with the Mst1 acetyltransferase by yeast-2-hybrid analysis 16. Despite this, any physical interaction between Mst1 and Msc1 is likely transient, as Msc1.TAP purification did not identify Mst1 (or any non-shared subunit of the Swr1 and Mst1 Complexes: Supplementary Table 1), and unique members of the Mst1-C do not co-purify Msc1 (not shown). However this is an interesting link between the Swr1 and Mst1 complexes, and may suggest that Msc1 directly recruits Mst1 to sites of Pht1 integration.

### N-terminal acetylation is integral to Pht1 function

The Swr-C is required for the assembly of Pht1 into chromatin, where it is acetylated by Mst1 (Figs. 1 and 2). This relationship between the ATPase (Swr1), histone variant (H2A.Z) and KAT5-family acetyltransferase (Mst1) appears to be widely conserved 9·15·43–46. Unacetyltable *pht1* phenocopies *pht1Δ* throughout this work, but is perhaps most striking in large-scale gene expression and genetic analyses (Fig. 3). This strongly suggests that acetylation of the histone is integral to its function. Based on *Sc* Htz1, it is likely that all four lysine residues in the Pht1 N-terminus are modified 10·11. However our *pht1*<sup>Ac</sup> antibody was raised to a tetra-acetylated peptide (Fig. 1a) and the unacetyltable *pht1* used throughout this work has mutations at all four N-terminal lysines, so this is as yet unconfirmed. *mst1*-

L271P reduces Pht1<sup>Ac</sup> below the threshold of detection (Fig. 1d), suggesting we have identified the major enzyme for this modification. In this regard, we note that *mst1* mutants also show increased rates of chromosome loss 16, likely due in part to reduced Pht1<sup>Ac</sup>.

### Pht1 regulates chromosome architecture at anaphase

Knockout or depletion of H2A.Z in *Sc* 15, *Sp* (this work and 25·26), or mammalian cells 5 leads to increased rates of chromosome loss. An acetylated form of H2A.Z mediates the chromosome stability role in both *Sc* 9 and *Sp* (this work), strongly suggesting that this will prove to be the case in other organisms. Directly monitoring chromosome segregation in individual *Sp* cells allowed us to show that lack of Pht1 induces chromosome arm entanglements in anaphase that can lead to chromosome breaks (Fig. 4). Furthermore, Pht1 is required for the stable association of condensin with chromatin through anaphase (Fig. 5d), and the chromosome entanglement in *pht1Δ* cells can be rescued by a pre-anaphase arrest where chromosomes hyper-condense (Fig. 5b). Thus the lack of Pht1 interferes with chromosome architecture in anaphase. However it also improves the viability of mutants that regulate this process, such as those in *condensin* (*cut3-477*, *cut14-208*, *cnd2-1*) or topoisomerase II (*top2-191*) (Fig. 5c and Supplementary Fig. 4). To resolve this apparent contradiction, we propose that Pht1 actually plays a dual role in mitotic chromosome architecture (see below).

Mitotic defects in a chicken *condensin* mutant primarily occur in anaphase, such that chromosomes prematurely lose their compact organization as they move to the poles 47. Thereafter individual chromatids can no longer be distinguished and prominent chromatin bridges are visible in >90% of cells (e.g. Fig. 6b and Supplementary Fig. 3a). These phenotypes can be overcome if Repo-Man, a targeting subunit for protein phosphatase 1 (PP1), is unable to direct the phosphatase to chromosomes in anaphase 47. In WT cells Repo-Man is subject to CDK-dependent phosphorylation(s) to abrogate chromosome binding at early mitosis when CDK levels are high. Chromosome compaction at this stage is only slightly affected in *condensin* mutants, prompting the Earnshaw lab to propose that an as-yet-uncharacterized activity, Regulator of Chromosome Architecture (RCA), drives initial condensation. RCA is then inhibited in a Repo-Man/PP1-dependent manner after CDK levels fall at anaphase onset and condensin steps in to stabilize chromosome architecture until mitosis completes 47 (all modeled in Fig. 6d).

The above model suggests that there are at least two steps in chromosome condensation. The first is RCA-dependent compaction to the characteristic X-shaped mitotic chromosome. The second is the condensin-dependent maintenance of a robust architecture that can withstand the pulling forces of microtubules in anaphase. Our data suggests that Pht1 regulates both steps: promoting the inhibition of RCA, and stabilizing the association of condensin. As above, artificially maintaining RCA activity in anaphase by preventing the loading of PP1 to chromatin partly rescues the chromosome segregation defects of chicken *smc2* mutants 47. This is reminiscent of the genetic interactions between *pht1* and *condensin* (e.g. Fig. 5c). It is highly likely that RCA is conserved in *Sp* as deletion of PP1<sup>Dis2</sup>, the fission yeast PP1 ortholog that localizes on chromatin, also partly rescues *cut3-477* (Supplementary Fig. 4b). Finally we have recently shown that *Sc* Bud14, a regulator of *Sc* PP1 (Glc7), is required for the efficient loading of Htz1 onto chromatin (and its subsequent acetylation) 48. This suggests that H2A.Z could act downstream of PP1 to inhibit RCA in anaphase (Fig. 6d). Further studies are underway to resolve this model.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.



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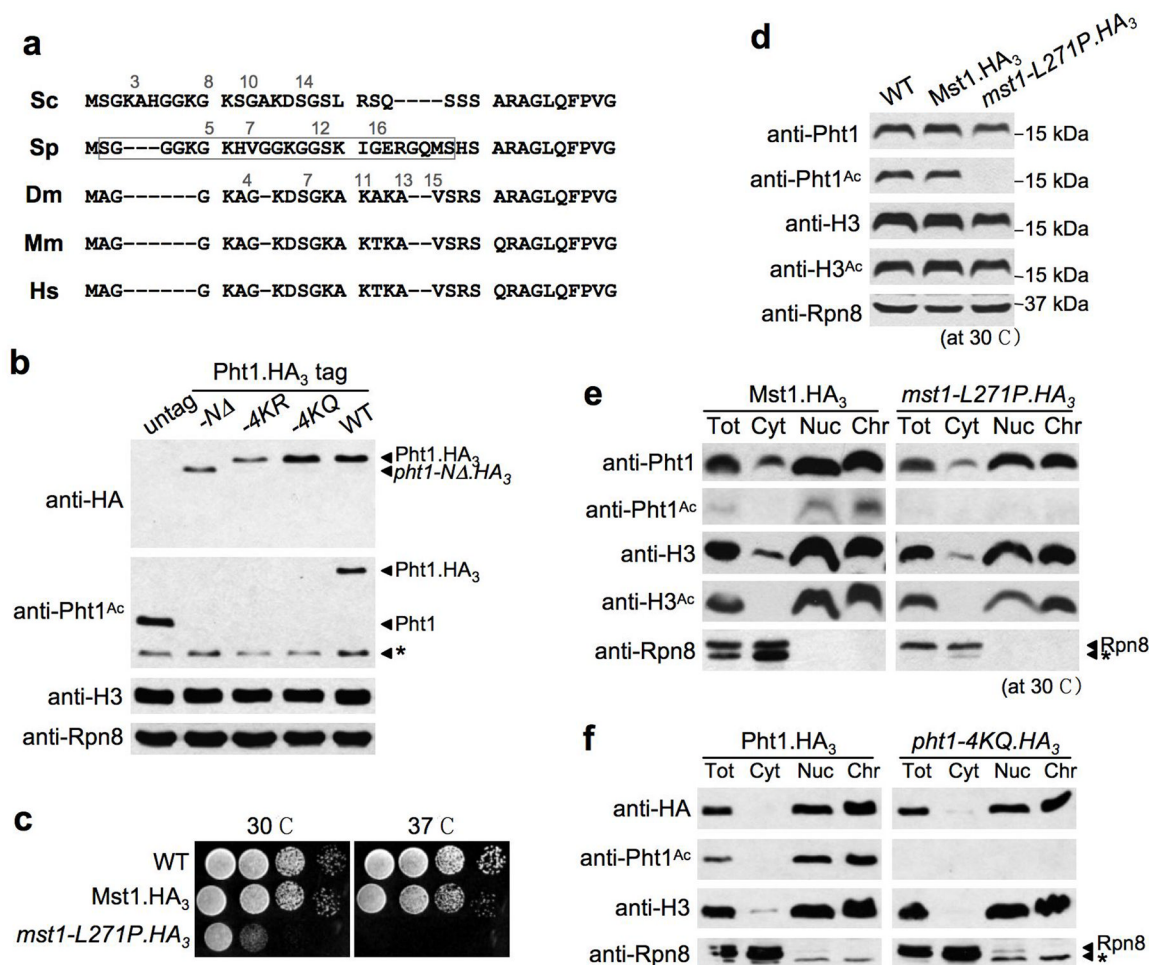
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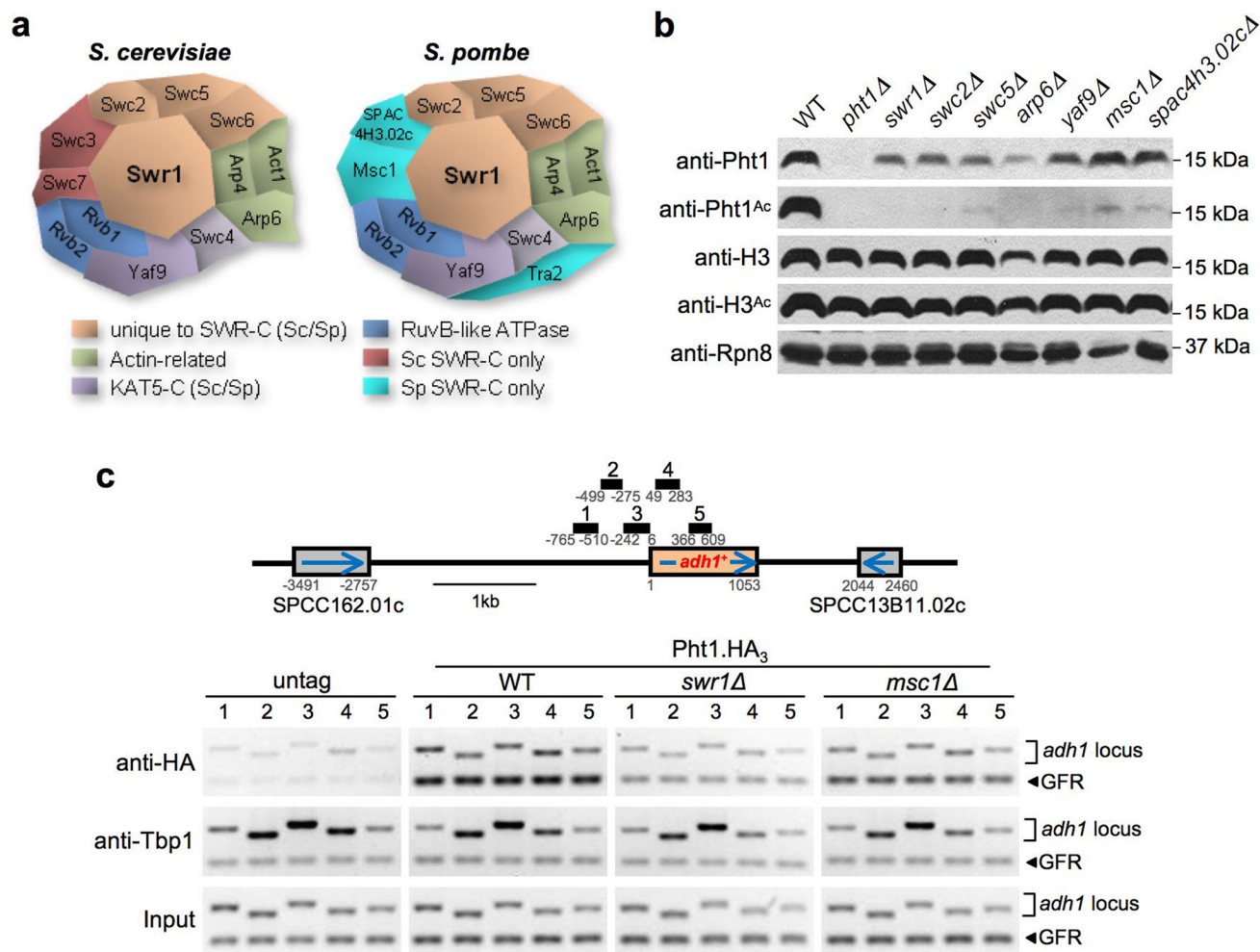
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**Figure 1. Chromatin-associated Pht1 is acetylated by Mst1**

(a) Alignment of the H2A.Z N-termini from *Sc* (Htz1, YOL012C), *Sp* (Pht1, SPBC11B10.10c), *Drosophila melanogaster* (*Dm*; H2Av, P028985), *Mus musculus* (*Mm*; NP\_058030) and *Homo sapiens* (*Hs*; NP\_002097). Pht1 is mis-annotated in the literature by the addition of 32 N-terminal residues (MILRHAPRVHESAFSLTHKTF AFCNCNNRFRKM-). This would give a protein of 18kDa rather than the 14kDa observed. The acetylated residues on *Sc* Htz1 (K3, 8, 10 and 14) correspond to *Sp* Pht1 K5, 7, 12 and 16. A synthetic tetra-Ac peptide covering this region (boxed) was immunized into rabbits to create anti-Pht1<sup>Ac</sup>. (b) Pht1 is acetylated on the N-terminus. Immunoblotting was performed on strains expressing C-terminally HA<sub>3</sub>-tagged Pht1 (or indicated mutants) at the endogenous locus. Total Pht1 was detected by anti-HA (12CA5; Supplementary Table 3). \* in anti-Pht1<sup>Ac</sup> panel refers to cross-reaction with H4<sup>Ac</sup>. H3 and Rpn8 serve as loading controls. (c) The *mst1-L271P* allele is slow and *ts*. Spot tests are 10-fold dilutions onto YES plates. (d) WCEs from *mst1-L271P* show a profound reduction in Pht1<sup>Ac</sup> without affecting total Pht1 or H3<sup>Ac</sup> levels. H3 and Rpn8 serve as loading controls. (e – f) Pht1 acetylation by Mst1 is not required for assembly of the histone into chromatin. In f strains express Pht1.HA<sub>3</sub> or unacetylatable *pht1-4KQ*.HA<sub>3</sub> at the endogenous locus. Cells were spheroblasted (Total) and fractionated into Cytoplasm, Nucleus and Chromatin. H3 and Rpn8 controls (\* cross-reacting species) indicate efficient fractionation: the former is primarily localized in insoluble chromatin, the latter in soluble cytoplasm.

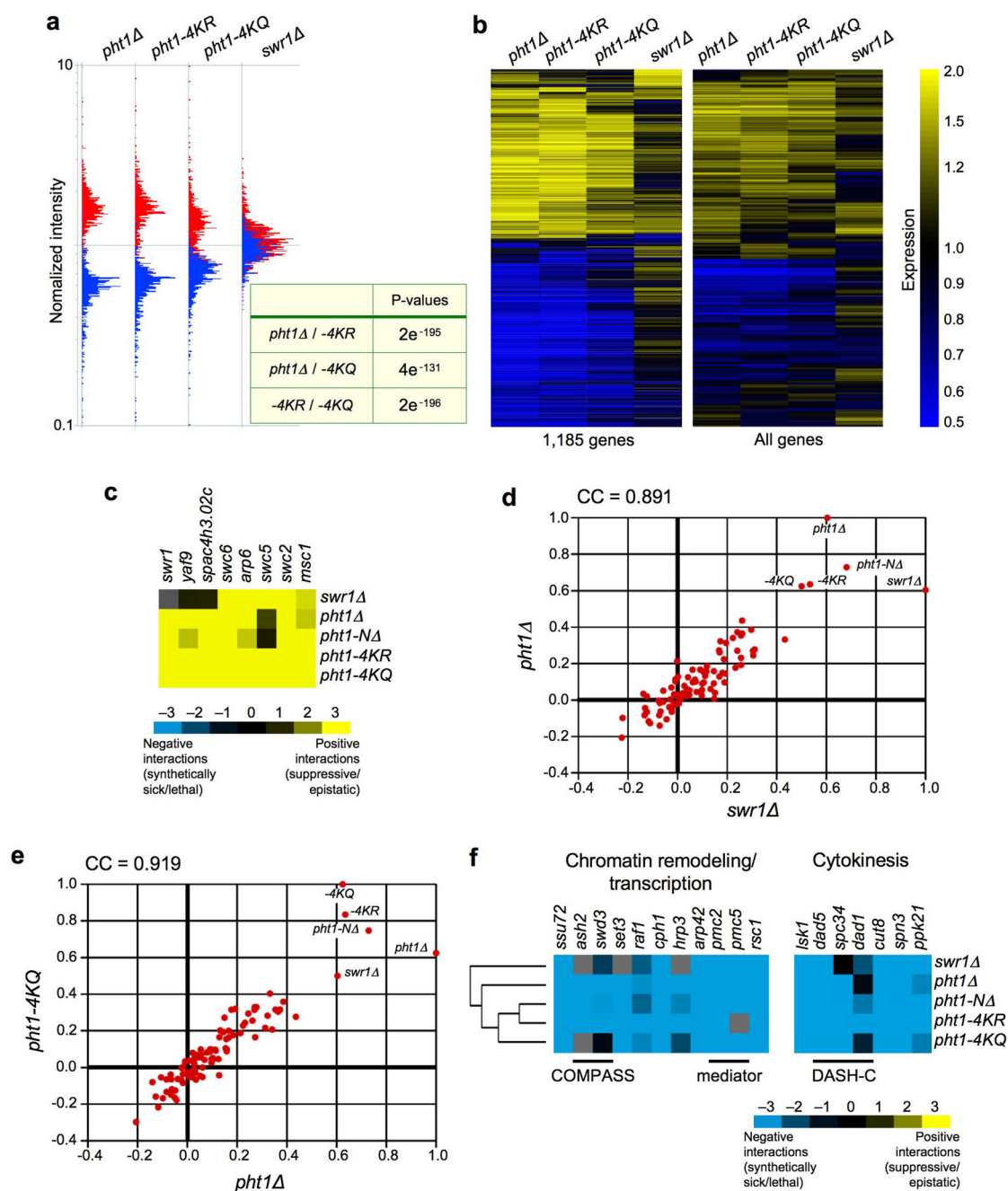




**Figure 2. Pht1 is inserted into chromatin by the SWR-C**

(a) The SWR-C is highly conserved from *Sp* to *Sc*. Multiple subunits of each complex were C-terminally TAP-tagged and associated factors identified by MS (see Supplementary Table 1 and 17). In this schematic location does not indicate direct interaction. (b) SWR-C components are individually required for the efficient acetylation of Pht1, suggesting chromatin-insertion defects. Total Pht1 levels are also reduced in many of these mutants. Immunoblotting of H3, H3Ac and Rpn8 serve as loading controls. (c) Pht1 is inefficiently assembled to chromatin in *swr1Δ*, and to a lesser extent, *msc1Δ*. Schematic depicts the neighborhood of constitutively expressed *adh1*<sup>+</sup>. Black boxes depict the location of ChIP primers (Supplementary Table 5) relative to the start codon (+1). Pht1.HA<sub>3</sub> was detected by anti-HA. Appropriate localization of Tbp1 at the *adh1*<sup>+</sup> promoter confirms sample integrity. In these duplex reactions upper band (*adh1* locus) are the specific primers numbered in the schematic, lower band (GFR) is a non-transcribed Gene-Free Region included as a loading control. Lower panel (Input) is used to normalize the PCR amplification efficiency of each primer pair.

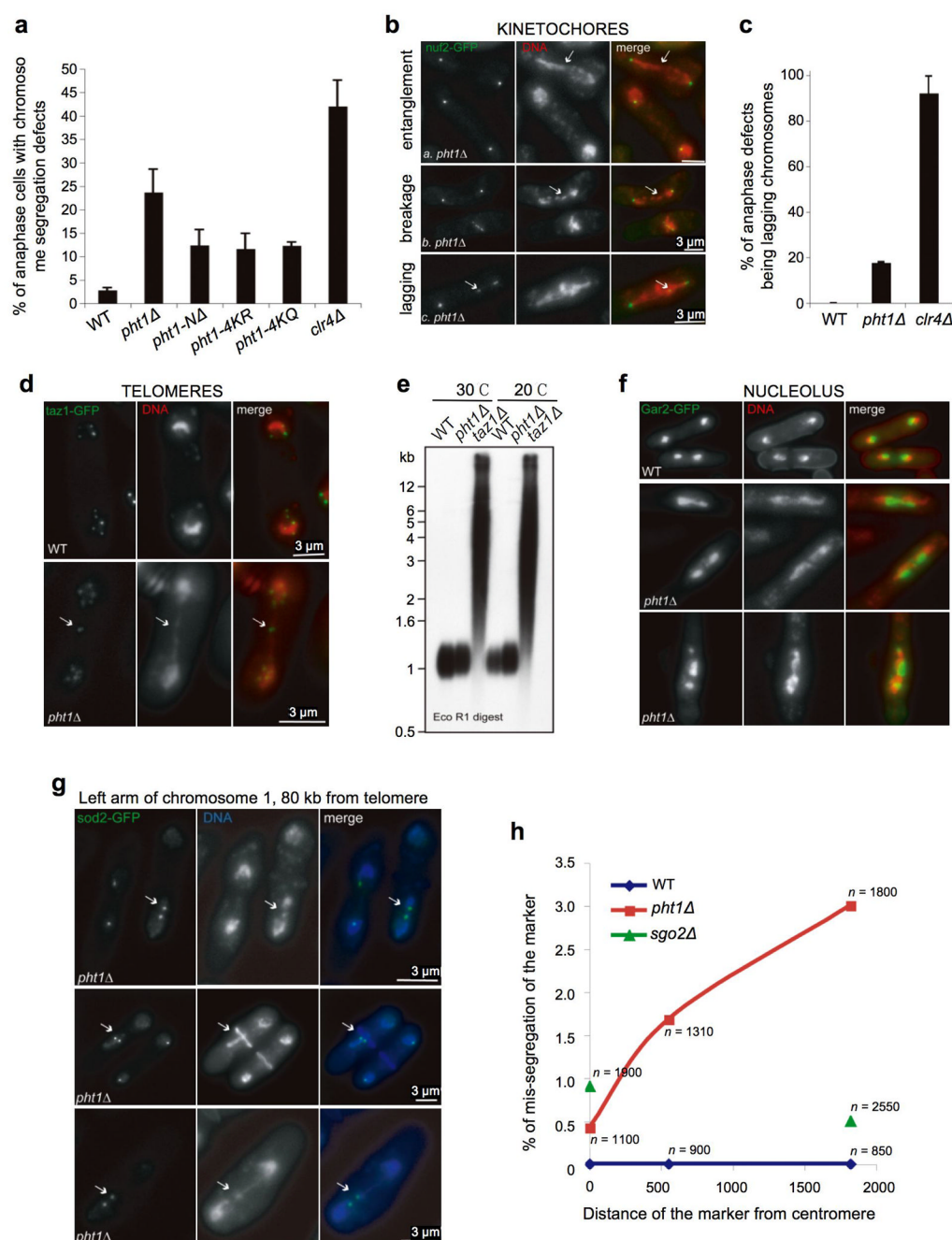




**Figure 3. Unacetyltable *pht1* mutants phenocopy *pht1Δ***

(a) Panel compares induced (red) and repressed (blue) genes (>1.5-fold in at least one condition) for whole genome array data from each strain. Relative P-values for expression overlaps among induced genes between strain pairs are as indicated. (b) Heat map of induced (yellow) and repressed (blue) genes in each mutant. Left: 1,185 genes are >1.5-fold changed in at least one mutant background. Right: all genes on the array. (c) Unacetyltable *pht1* mutants display positive genetic interactions with deletions of SWR-C subunits. (d) Correlation coefficient (CC) plot comparing the genetic interactions of 101 query mutants (including *pht1Δ*, -NΔ, -4KR, -KQ and *swr1Δ*) mated against a library of 2,161 non-essential deletions. Red dots indicate the CC of each genetic screen to the mutants on the X-

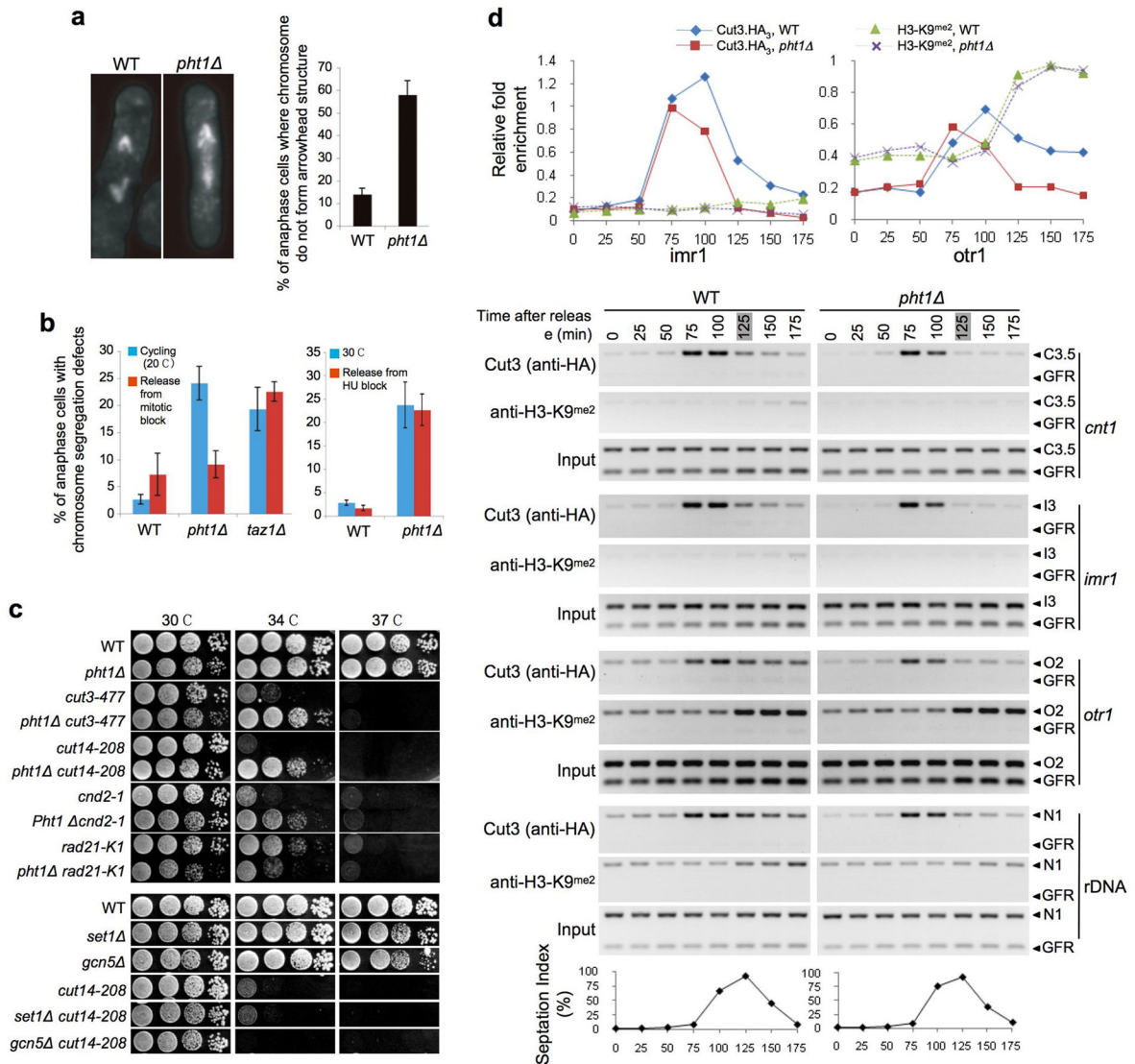
or Y-axes. **(e)** CC plot of *pht1*Δ Vs *pht1-4KQ*. **(f)** *pht1*Δ, *-N*Δ, *-4KR*, *-KQ* and *swr1*Δ share a large number of synthetic genetic interactions, including with members of the COMPASS, RSC, SET3-C, Mediator and DASH complexes.



**Figure 4. Lack of Pht1<sup>Ac</sup> leads to chromosome segregation defects in anaphase**

(a) The percentage (mean  $\pm$  s.d.) of late anaphase cells with chromosome segregation defects (see succeeding panels) was visually scored. All strains were grown in YES at 30°C. (b) Kinetochore segregation was monitored by distribution of Nuf2-GFP 49. Three chromosome mis-segregation defects (arrowed) are distinguished in *pht1Δ* anaphase cells: lagging, entanglement, and entanglement leading to breakage. (c) The primary chromosome segregation defect in *pht1Δ* (entanglement) is distinct from that in heterochromatin-deficient *clr4Δ* (lagging). Bars are mean  $\pm$  s.d. (d) Telomere segregation was monitored by distribution of Taz1-GFP 29. In the example two individual telomeres (two Taz1 spots) are entangled in the middle of a *pht1Δ* anaphase cell, leading to stretched chromosome arms. (e)

Pht1 (in contrast to Taz1) does not regulate telomere repeat length. **(f)** Nucleolar segregation was monitored by distribution of Gar2-GFP 50. In many *pht1* $\Delta$  cells the nucleolus appears stretched or fragmented. **(g – h)** Chromosome marker loss in *pht1* $\Delta$  cells increases with distance from the centromere. GFP-marked loci locations (*cen2-lacO*, 5 kb from CEN2; *ade3-lacO*, 1350 kb from TEL1-L; *sod2-lacO*, 80 kb from TEL1-L 30) were used to monitor and quantify chromosome segregation defects. *n* represents the number of binucleate cells counted for each marker in each background.

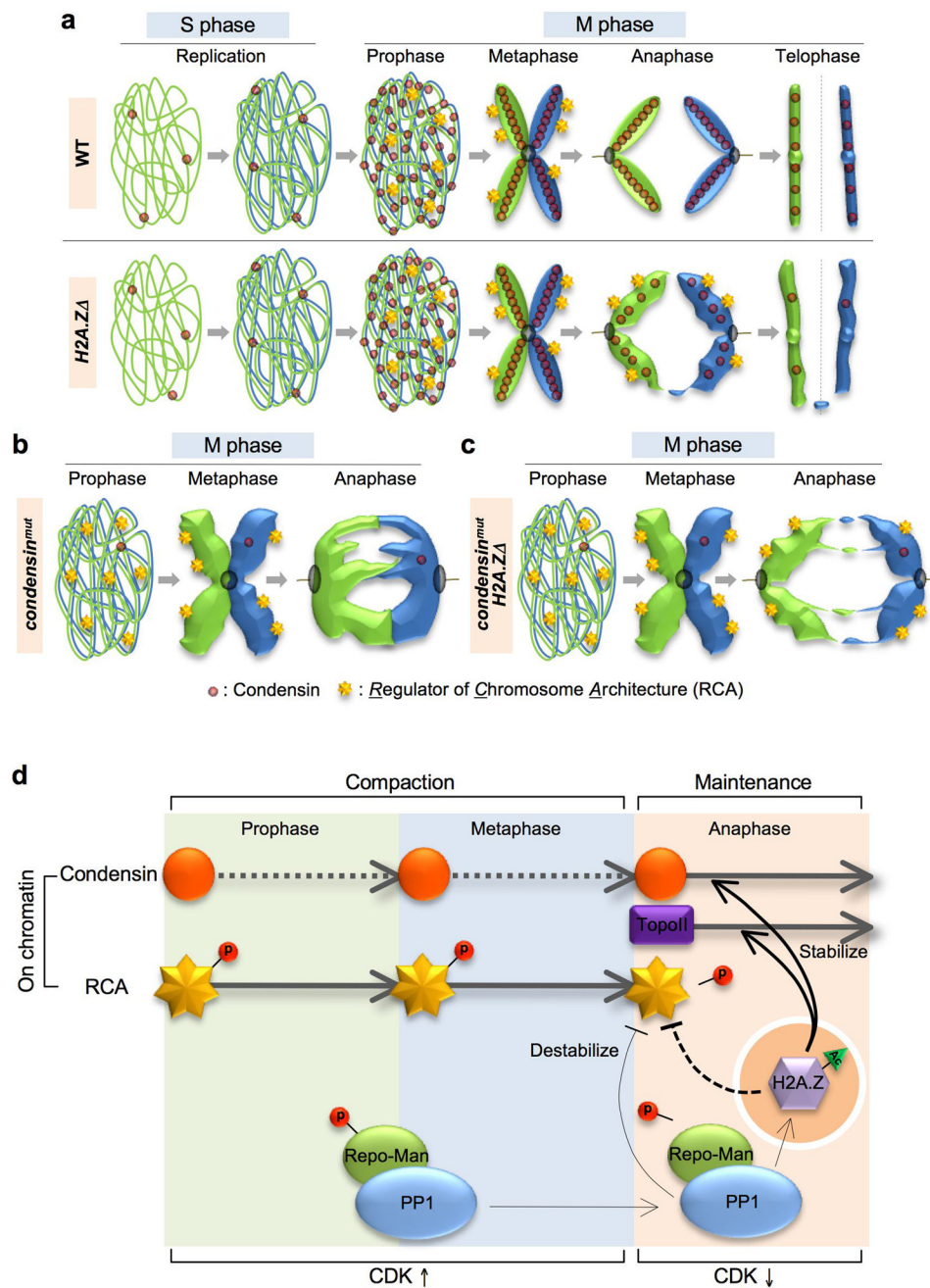


### Figure 5. Pht1 plays a role in chromosome architecture/compaction

**(a)** *pht1Δ* (and  $-4KR$  or  $-4KQ$ , not shown) cells frequently (mean  $\pm$  s.d.) lose the “arrowhead” structure of segregating chromosomes in anaphase, indicating a disruption of chromatin architecture. In the example each anaphase (WT and *pht1Δ*) is at roughly the same stage: 6.5  $\mu$ m between the poles. **(b)** The chromosome segregation defects of *pht1Δ*, but not *taz1Δ*, can be rescued by chromosome hyper-condensation induced by prolonged mitotic arrest. WT, *pht1Δ* or *taz1Δ* cells in the background of a cold-sensitive tubulin mutant *nda3-KM311* (red) or not (blue) were placed at the restrictive temperature (20°C, six hours), during which *nda3-KM311* fails to assemble microtubules and undergoes spindle-dependent checkpoint arrest. Cells were released into anaphase at the permissive temperature (32°C) and scored for chromosome segregation defects. Arresting with HU has no effect on the phenotype. **(c)** *pht1Δ* partially rescues mutants in three subunits of *condensin* (each SMC and the kleisin: *cut3-477*, *cut14-208* and *cnd2-1*), but is synthetic with a mutant in *cohesin* (kleisin: *rad21-K1*). The ability to rescue *condensin* is not shared by deletions of the Set1 methyltransferase or Gcn5 acetyltransferase. Spot tests are 10-fold dilutions onto YES plates. **(d)** WT or *pht1Δ* (additionally containing Cut3.HA<sub>3</sub> and



*cdc25-22*) were arrested in G<sub>2</sub> and released into the cell cycle (with samples taken every 25 minutes as in Supplementary Methods). Septation index confirms that each population completes mitosis by 125 minutes (peak septation; shaded in grey). ChIP was used to monitor condensin and H3-K9<sup>Me2</sup> at various locations, including the Chr I centromere (for primer schematic see Supplementary Fig. 1c) and rDNA. While H3-K9<sup>Me2</sup> at *imr1* and *otr1* follows similar creation kinetics in WT and *pht1Δ*, Cut3.HA<sub>3</sub> drops prematurely at all locations in the absence of the histone variant (see also Supplementary Fig. 3d). The “Relative fold enrichment” in the ChIP quantitation graphs is the ratio between the specific signal at each location and the respective Input.



**Figure 6. H2A.Z plays a role in higher order chromosome architecture**

**(a). Upper row:** The localization/association of condensin with chromosomes is tightly regulated. The complex is primarily (but not exclusively) found in the cytoplasm through interphase, and imported into the nucleus at prophase for chromosome loading, with levels peaking at anaphase 51:52. **Lower row:** the pathway in *pht1Δ* cells. Condensin loads normally but prematurely dissociates (Fig. 5d). This explains the poorly resolved structure observed in anaphase (Fig. 5a), which likely leads to chromosome entanglement and loss (Fig. 4). **(b)** Sister chromatids in condensin mutants are “fuzzy” and resolve poorly at prophase. The sisters remain connected by chromatin bridges as they pull apart in anaphase (see Supplementary Fig. 3a). **(c)** The rescue by *pht1Δ* (and unacetyltable mutants) of

various chromosome architecture mutants (*condensin* and Topo II; Fig. 5c and Supplementary Fig. 3b and 4) indicates that a partially compensatory condensed topology exists in *pht1Δ* cells. **(d)** Model separates mitotic chromosome condensation/architecture into two stages: initial compaction and maintenance. The first stage is dependent on the poorly characterized activity (or factor) RCA, the second on the condensin complex and topoisomerase II. H2A.Z may have a dual regulatory role: somehow opposing RCA, yet stabilizing the association of condensin with chromatin.